IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Levitsky et al.

Art Unit: Unassigned

Application No. Unassigned

Examiner: Unassigned

Filed: November 16, 2001

For: A UNIVERSAL IMMUNOMODULATORY CYTOKINE-EXPRESSING BYSTANDER CELL LINE AND RELATED COMPOSITIONS AND METHODS OF MANUFACTURE AND USE

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to the examination of the above-identified patent application, please enter the following amendments and consider the following remarks.

AMENDMENTS

IN THE SPECIFICATION:

The paragraph beginning at page 20, line 12, has been amended to read:

As shown in Fig. 1, subclones of GM-CSF-producing K562 cells produced in excess of 1,000 ng/10^6 cells/24 hrs. The use of such subclones enables the use of as few as one bystander cell per 10 autologous tumor cells with a clear margin of safety above the GM-CSF threshold of 36 ng GM-CSF/10^6 cells/24 hrs, by targeting 100 ng/10^6 cells/24 hrs.

IN THE CLAIMS:

Please cancel claims 15, 16, 29-39, 48 and 49.

Please amend the indicated claims to read as follows:

1. A universal bystander cell line, which:
(i) is a human cell line,

(ii) naturally lacks major histocompatibility class I (MHC-I) antigens and major histocompatibility class II (MHC-II) antigens, and

(iii) is modified by introduction of a nucleic acid molecule comprising a nucleic acid sequence encoding granulocyte macrophage-colony stimulating factor (GM-CSF) operably linked to a promoter,

wherein said universal bystander cell line expresses about 500 ng or greater GM-CSF/10^6 cells/24 hours.

5. The universal bystander cell line of claim 1, which expresses about 1,000 ng or greater GM-CSF/10^6 cells/24 hours.

8. The universal bystander cell line of claim 4, which expresses about 1,000 ng or greater GM-CSF/10^6 cells/24 hours.

11. The universal bystander cell line of claim 1, wherein said nucleic acid molecule further comprises a nucleic acid sequence encoding hygromycin resistance operably linked to a promoter and said universal bystander cell line is selected by growth in a culture medium comprising about 400 μg/ml or greater hygromycin.

12. The universal bystander cell line of claim 11, wherein said universal bystander cell line is selected by growth in a culture medium comprising about 1,000 μg/ml or greater hygromycin.

13. The universal bystander cell line of claim 4, wherein said nucleic acid molecule further comprises a nucleic acid sequence encoding hygromycin resistance operably linked to a promoter and said universal bystander cell line is selected by growth in a culture medium comprising about 400 μg/ml or greater hygromycin.

14. The universal bystander cell line of claim 13, wherein said universal bystander cell line is selected by growth in a culture medium comprising about 1,000 μg/ml or greater hygromycin.

22. A method of making a universal GM-CSF-expressing bystander cell line, which method comprises:

(i) obtaining a human cell line that lacks MHC-I antigens and MHC-II antigens;

(ii) modifying said human cell line by introducing into said human cell line a nucleic acid molecule comprising a nucleic acid sequence encoding GM-CSF operably linked to a promoter and a nucleic acid sequence encoding a selectable marker operably linked to a promoter; and

(iii) using the selectable marker to isolate cells that produce about 500 ng or greater of said GM-CSF/10^6 cells/24 hours.

24. The method of claim 23, wherein the modified human cell line is cultured in culture medium comprising about 400 μg or greater hygromycin/ml culture medium.

25. The method of claim 24, wherein the modified human cell line is subsequently cultured in culture medium comprising about 1,000 μg or greater hygromycin/ml culture medium.

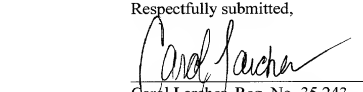
REMARKS

The specification has been amended at page 20, line 12, to change Fig. 4 to Fig. 1. This amendment corrects an obvious error in Example 2. Fig. 4 clearly does not show subclones of GM-CSF-producing K562 cells that produced in excess of 1,000 ng/10^6 cells/24 hours. Rather, Fig. 4 clearly presents a graph of percent tumor-free survival versus days post-tumor challenge. Because Fig. 1 shows GM-CSF-producing K562 cells that produced in excess of 1,000 ng/10^6 cells/24 hrs, Fig. 1 should be referenced in Example 2 at page 20, line 12, rather than Fig. 4. Because this amendment merely corrects an obvious error, this amendment has added no new subject matter to the specification.

The claims have been amended in view of the restriction requirement in the parent application. In addition, claims 1, 5, 8, 11-14 and 22-25 have been amended to point out more particularly and claim more distinctly the present invention by changing the phrase "at least about" to "about...or greater." No new matter has been added by way of these amendments.

The application is considered to be in good and proper form for allowance and the Examiner is respectfully requested to pass this application to issuance. If, in the opinion of the Examiner, a telephone conference would expedite the examination of this application, the Examiner is invited to contact the undersigned attorney.

Respectfully submitted,



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Date: November 16, 2001

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AMENDMENTS TO SPECIFICATION AND CLAIMS MADE VIA PRELIMINARY AMENDMENT

Amendments to the paragraph beginning at page 20, line 12:

As shown in [Fig. 4] Fig. 1, subclones of GM-CSF-producing K562 cells produced in excess of 1,000 ng/10^6 cells/24 hrs. The use of such subclones enables the use of as few as one bystander cell per 10 autologous tumor cells with a clear margin of safety above the GM-CSF threshold of 36 ng GM-CSF/10^6 cells/24 hrs, by targeting 100 ng/10^6 cells/24 hrs.

Amendments to existing claims:

Claims 15, 16, 29-39, 48 and 49 have been canceled.

The indicated claims have been amended as follows:

1. (Amended) A universal bystander cell line, which:

(i) is a human cell line,

(ii) naturally lacks major histocompatibility class I (MHC-I) antigens and major histocompatibility class II (MHC-II) antigens [or is modified so that it lacks MHC-I antigens and MHC-II antigens], and

(iii) is modified by introduction of a nucleic acid molecule comprising a nucleic acid sequence encoding granulocyte macrophage-colony stimulating factor (GM-CSF) operably linked to a promoter,

wherein said universal bystander cell line expresses [at least] about 500 ng or greater GM-CSF/10^6 cells/24 hours.

5. (Amended) The universal bystander cell line of claim 1, which expresses [at least] about 1,000 ng or greater GM-CSF/10^6 cells/24 hours.

8. (Amended) The universal bystander cell line of claim 4, which expresses [at least] about 1,000 ng or greater GM-CSF/10^6 cells/24 hours.

11. (Amended) The universal bystander cell line of claim 1, wherein said nucleic acid molecule further comprises a nucleic acid sequence encoding hygromycin resistance operably linked to a promoter and said universal bystander cell line is selected by growth in a culture medium comprising [at least] about 400 μg/ml or greater hygromycin.

12. (Amended) The universal bystander cell line of claim 11, wherein said universal bystander cell line is selected by growth in a culture medium comprising [at least] about 1,000 μg/ml or greater hygromycin.

13. (Amended) The universal bystander cell line of claim 4, wherein said nucleic acid molecule further comprises a nucleic acid sequence encoding hygromycin resistance operably linked to a promoter and said universal bystander cell line is selected by growth in a culture medium comprising [at least] about 400 μg/ml or greater hygromycin.

14. (Amended) The universal bystander cell line of claim 13, wherein said universal bystander cell line is selected by growth in a culture medium comprising [at least] about 1,000 μg/ml or greater hygromycin.

22. (Amended) A method of making a universal GM-CSF-expressing bystander cell line, which method comprises:

(i) obtaining a human cell line that lacks MHC-I antigens and MHC-II antigens;

(ii) modifying said human cell line by introducing into said human cell line a nucleic acid molecule comprising a nucleic acid sequence encoding GM-CSF operably linked to a promoter and a nucleic acid sequence encoding a selectable marker operably linked to a promoter; and

(iii) using the selectable marker to isolate cells that produce [at least] about 500 ng or greater of said GM-CSF/10^6 cells/24 hours.

24. (Amended) The method of claim 23, wherein the modified human cell line is cultured in culture medium comprising [at least] about 400 μg or greater hygromycin/ml culture medium.

25. (Amended) The method of claim 24, wherein the modified human cell line is subsequently cultured in culture medium comprising [at least] about 1,000 μg or greater hygromycin/ml culture medium.

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PENDING CLAIMS AFTER ENTRY OF PRELIMINARY AMENDMENT

1. A universal bystander cell line, which:

(i) is a human cell line,

(ii) naturally lacks major histocompatibility class I (MHC-I) antigens and major histocompatibility class II (MHC-II) antigens, and

(iii) is modified by introduction of a nucleic acid molecule comprising a nucleic acid sequence encoding granulocyte macrophage-colony stimulating factor (GM-CSF) operably linked to a promoter,

wherein said universal bystander cell line expresses about 500 ng or greater GM-CSF/10^6 cells/24 hours.

2. The universal bystander cell line of claim 1, wherein said human cell line is characterized by the absence of B-lymphocyte markers of immunoglobulin, an Epstein-Barr virus (EBV) genome and an associated nuclear antigen, and receptors for EBV.

3. The universal bystander cell line of claim 1, wherein said human cell line is derived from a blast crisis of chronic myeloid leukemia.

4. The universal bystander cell line of claim 1, wherein said human cell line is K562.

5. The universal bystander cell line of claim 1, which expresses about 1,000 ng or greater GM-CSF/10^6 cells/24 hours.

6. The universal bystander cell line of claim 1, which grows in defined medium.

7. The universal bystander cell line of claim 1, wherein said promoter is a cytomegalovirus promoter.

8. The universal bystander cell line of claim 4, which expresses about 1,000 ng or greater GM-CSF/10^6 cells/24 hours.

9. The universal bystander cell line of claim 4, which grows in defined medium.

10. The universal bystander cell line of claim 4, wherein said promoter is a cytomegalovirus promoter.

11. The universal bystander cell line of claim 1, wherein said nucleic acid molecule further comprises a nucleic acid sequence encoding hygromycin resistance operably linked to a promoter and said universal bystander cell line is selected by growth in a culture medium comprising about 400 μg/ml or greater hygromycin.

12. The universal bystander cell line of claim 11, wherein said universal bystander cell line is selected by growth in a culture medium comprising about 1,000 μg/ml or greater hygromycin.

13. The universal bystander cell line of claim 4, wherein said nucleic acid molecule further comprises a nucleic acid sequence encoding hygromycin resistance operably linked to a promoter and said universal bystander cell line is selected by growth in a culture medium comprising about 400 μg/ml or greater hygromycin.

14. The universal bystander cell line of claim 13, wherein said universal bystander cell line is selected by growth in a culture medium comprising about 1,000 μg/ml or greater hygromycin.

17. A composition comprising the universal bystander cell line of claim 1 and a cancer antigen.

18. A composition comprising the universal bystander cell line of claim 2 and a cancer antigen.

19. A composition comprising the universal bystander cell line of claim 4 and a cancer antigen.

20. A composition comprising the universal bystander cell line of claim 5 and a cancer antigen.

21. A composition comprising the universal bystander cell line of claim 8 and a cancer antigen.

22. A method of making a universal GM-CSF-expressing bystander cell line, which method comprises:

(i) obtaining a human cell line that lacks MHC-I antigens and MHC-II antigens;

(ii) modifying said human cell line by introducing into said human cell line a nucleic acid molecule comprising a nucleic acid sequence encoding GM-CSF operably linked to a promoter and a nucleic acid sequence encoding a selectable marker operably linked to a promoter; and

(iii) using the selectable marker to isolate cells that produce about 500 ng or greater of said GM-CSF/10^6 cells/24 hours.

23. The method of claim 22, wherein said selectable marker is hygromycin resistance.

24. The method of claim 23, wherein the modified human cell line is cultured in culture medium comprising about 400 μg or greater hygromycin/ml culture medium.

25. The method of claim 24, wherein the modified human cell line is subsequently cultured in culture medium comprising about 1,000 μg or greater hygromycin/ml culture medium.

26. The method of claim 24, wherein said culture medium is defined.

27. The method of claim 25, wherein said culture medium is defined.

28. The method of claim 22, wherein the promoter to which the nucleic acid sequence encoding GM-CSF is operably linked is a cytomegalovirus promoter.

40. A method of stimulating an immune response to a cancer in a human patient, which method comprises administering to said patient the composition of claim 17, wherein said cancer antigen is an antigen of said cancer and wherein said composition is irradiated,

whereupon administration of said composition, an immune response to said cancer is stimulated.

41. The method of claim 40, wherein said cancer antigen is a cell of said cancer.

42. A method of stimulating an immune response to a cancer in a human patient, which method comprises administering to said patient the composition of claim 19, wherein said cancer antigen is an antigen of said cancer and wherein said composition is irradiated,

whereupon administration of said composition, an immune response to said cancer is stimulated.

43. The method of claim 42, wherein said cancer antigen is a cell of said cancer.

44. A method of stimulating an immune response to a cancer in a human patient, which method comprises administering to said patient the composition of claim 20, wherein said cancer antigen is an antigen of said cancer and wherein said composition is irradiated,

whereupon administration of said composition, an immune response to said cancer is stimulated.

45. The method of claim 44, wherein said cancer antigen is a cell of said cancer.

46. A method of stimulating an immune response to a cancer in a human patient, which method comprises administering to said patient the composition of claim 21, wherein said cancer antigen is an antigen of said cancer and wherein said composition is irradiated,

whereupon administration of said composition, an immune response to said cancer is stimulated.

47. The method of claim 46, wherein said cancer antigen is a cell of said cancer.

50. In a method of cancer immunotherapy, the improvement comprising administering to a human patient having a cancer the composition of claim 17, wherein said cancer antigen is an antigen of said cancer and wherein said composition is irradiated.

51. In a method of cancer immunotherapy, the improvement comprising administering to a human patient having a cancer the composition of claim 19, wherein said cancer antigen is an antigen of said cancer and wherein said composition is irradiated.

52. In a method of cancer immunotherapy, the improvement comprising administering to a human patient having a cancer the composition of claim 20, wherein said cancer antigen is an antigen of said cancer and wherein said composition is irradiated.

53. In a method of cancer immunotherapy, the improvement comprising administering to a human patient having a cancer the composition of claim 21, wherein said cancer antigen is an antigen of said cancer and wherein said composition is irradiated.